Attorney Docket No. 04853.0138-00000

Application No.: 10/594,417

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the

application:

(Cancelled) 1.

2. (Currently Amended) The transformant according to claim 11, wherein the genes each-

encoding a subunit of glycerol dehydratase and/or diol dehydratase are derived from

Lactobacillus reuteri.

(Currently Amended) The transformant according to claim 11:, wherein which comprises 3.

the gene encoding propanol dehydrogenase is a gene encoding 1,3-propanediol oxidoreductase

and said gene is derived from Lactobacillus reuteri.

(Currently Amended) The transformant according to claim 11, which comprises wherein 4

the gene encoding propanol dehydrogenase 1.3-propanediol oxidoreductase and said gene is-

derived from Lactobacillus reuteri.

5. (Currently Amended) The transformant according to claim 11, wherein the genes

encoding the subunits each encoding a subunit of the reactivation factor for glycerol dehydratase

and/or the reactivation factor for diol dehydratase are derived from Lactobacillus reuteri.

6. (Currently Amended) The transformant according to claim 11, wherein the genes-

encoding aldehyde dehydrogenase are genes encoding propionaldehyde dehydrogenase, and said-

transformant further comprises the genes further comprising a gene encoding

3

Attorney Docket No. 04853.0138-00000 Application No.: 10/594,417

phosphotransacylase the genes and a gene encoding propionate kinase, but does not eomprise any gene encoding glycerol dehydrogenase.

- (Original) The transformant according to claim 6, which comprises the pdu operon, and no gene encoding glycerol dehydrogenase.
- 8. (Currently Amended) Knockout bacteria of Lactobacillus reuteri lacking glycerol dehydrogenase activity, which are obtained by knocking out the gene encoding glycerol dehydrogenase, from bacteria of the genera Lactobacillus, Salmonella, Klebsiella, Listeria, Clostridium, Escherichia, Enterobacter, Caloramator, Acetobacterium, Brucella, Flavobacterium, Fusobacterium, Citrobacter, or Propionibacterium.
- (Currently Amended) Knockout bacteria of <u>Lactobacillus reuteri</u> comprising the pdu
 operon and the <u>a</u> gene encoding phosphotransacylase, wherein-but not the gene encoding
 glycerol dehydrogenase, is knocked out.
- (Currently Amended) A method for producing 1,3-propanediol and/or 3hydroxypropionic acid by comprising:
- (a) bringing obtaining the transformants or bacteria according to any one of claims claim 11, to 9 into contact with glycerol,
- (b) culturing the transformant in the presence of glycerol, and
- (c) purifying the 1,3-propanediol and/or 3-hydroxypropionic acid.

 (New) A transformant of E. coli or Lactobacillus reuteri comprising genes encoding: large, medium, and small subunits of glycerol dehydratase and/or large, medium, and small subunits of diol dehydratase;

large and small subunits of a reactivation factor for glycerol dehydratase and/or large and small subunits of a reactivation factor for diol dehydratase;

propionaldehyde dehydrogenase; and

propanol dehydrogenase.

- (New) A method for producing 1,3-propanediol and/or 3-hydroxypropionic acid comprising:
- (a) obtaining the knockout bacteria of claim 8,
- (b) culturing the knockout bacteria in the presence of glycerol, and
- (c) purifying the 1,3-propanediol and/or 3-hydroxypropionic acid.
- 13. (New) A method for producing 1,3-propanediol and/or 3-hydroxypropionic acid comprising:
- (a) obtaining the knockout bacteria of claim 9,
- (b) culturing the bacteria in the presence of glycerol, and
- (c) purifying the 1,3-propanediol and/or 3-hydroxypropionic acid.
- 14. (New) The transformant according to claim 11, wherein the gene encoding the large subunit of glycerol dehydratase encodes the following protein (a) or (b):

- (a) a protein comprising an amino acid sequence as shown in SEQ ID NO: 1 or 3, or
- (b) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 1 or 3 by deletion, substitution, or addition of one or 2-3 amino

acid residues and having glycerol dehydratase activity when expressed with the medium

and small subunits of glycerol dehydratase;

wherein the gene encoding the medium subunit of glycerol dehydratase encodes the following protein (c) or (d):

- (c) a protein comprising an amino acid sequence as shown in SEQ ID NO: 5 or 7, or
- (d) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 5 or 7 by deletion, substitution, or addition of one or 2-3 amino acid residues and having glycerol dehydratase activity when expressed with the large and

small subunits of glycerol dehydratase; and

wherein the gene encoding the small subunit of glycerol dehydratase encodes the following protein (e) or (f):

- (e) a protein comprising an amino acid sequence as shown in SEQ ID NO: 9 or 11, or
- (f) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 9 or 11 by deletion, substitution, or addition of one or 2-3 amino

acid residues and having glycerol dehydratase activity when expressed with the large and

medium subunits of glycerol dehydratase.

(New) The transformant according to claim 11, 15.

Attorney Docket No. 04853.0138-00000 Application No.: 10/594,417

- wherein the gene encoding the large subunit of glycerol dehydratase comprises a nucleotide sequence as shown in SEQ ID NO: 2 or 4 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEQ ID NO: 2 or 4;
- wherein the gene encoding the medium subunit of glycerol dehydratase comprises a nucleotide sequence as shown in SEQ ID NO: 6 or 8 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEQ ID NO: 6 or 8; and
- wherein the gene encoding the small subunit of glycerol dehydratase comprises a nucleotide sequence as shown in SEQ ID NO: 10 or 12 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEO ID NO: 10 or 12.
- 16. (New) The transformant according to claim 11, wherein the gene encoding propanol dehydrogenase encodes the following protein (a) or (b):
 - (a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 13 or 15; or
 (b) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 13 or 15 by deletion., substitution, or addition of one or 2-3 amino acid residues and having propanol dehydrogenase activity.
- 17. (New) The transformant according to claim 11, wherein the gene encoding propanol dehydrogenase comprises a nucleotide sequence as shown in SEQ ID NO: 14 or 16 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEQ ID NO: 14 or 16.
- 18. (New) The transformant according to claim 11,

Attorney Docket No. 04853.0138-00000 Application No.: 10/594,417

wherein the gene encoding propanol dehydrogenase is a gene encoding 1,3-propanediol oxidoreductase that encodes the following protein (a) or (b):

- (a) a protein comprising an amino acid sequence as shown in SEQ ID NO: 17; or
- (b) a protein comprising an amino acid sequence derived from the ammo acid sequence as shown in SEQ ID NO: 17 by deletion, substitution, or addition of one or 2-3 amino acid residues and having 1,3-propanediol oxidoreductase activity.
- 19. (New) The transformant according to claim 11, wherein the gene encoding propanol dehydrogenase is a gene encoding 1,3-propanediol oxidoreductase comprising a nucleotide sequence as shown in SEQ ID NO: 18 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEQ ID NO: 18.
- 20. (New) The transformant according to claim 11, wherein the gene encoding the large subunit of the reactivation factor for glycerol dehydratase encodes the following protein (a) or (b):
 - (a) a protein comprising an amino acid sequence as shown in SEQ ID NO: 19 or 21; or
 (b) a protein comprising an amino acid sequence derived from the amino acid sequence
 as shown in SEQ ID NO: 19 or 21 by deletion, substitution, or addition of one or 2-3
 amino acid residues and having the activity of the reactivation factor for glycerol
 dehydratase when expressed with the small subunit of the reactivation factor for glycerol
 dehydratase, and

wherein the gene encoding the small subunit of the reactivation factor for glycerol dehydratase encodes the following protein (e) or (d):

- (c) a protein comprising an amino acid sequence as shown in SEQ ID NO: 23 or 25; or
 (d) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 23 or 25 by deletion, substitution, or addition of one or 2-3
- amino acid residues and having the activity of the reactivation factor for glycerol dehydratase when expressed with the large subunit of the reactivation factor for glycerol dehydratase.
- (New) The transformant according to claim 11,
- wherein the gene encoding the large subunit of the reactivation factor for glycerol dehydratase comprises a nucleotide sequence as shown in SEQ ID NO: 20 or 22 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEQ ID NO: 20 or 22; and
- wherein the gene encoding the small subunit of the reactivation factor for glycerol dehydratase comprises a nucleotide sequence as shown in SEQ ID NO: 24 or 26 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEQ ID NO: 24 or 26.
- 22. (New) The transformant according to claim 11, wherein the gene encoding propional dehyde dehydrogenase encodes the following protein (a) or (b):
 - (a) a protein comprising an amino acid sequence as shown in SEQ ID NO: 41; or

- (b) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 41 by deletion, substitution or addition of one or 2-3 amino acid residues and having propionaldehyde dehydrogenase activity.
- 23. (New) The transformant according to claim 11, wherein the gene encoding propionaldehyde dehydrogenase comprises a nucleotide sequence as shown in SEQ ID NO: 42 or a nucleotide sequence that hybridizes under stringent

conditions to a complement of SEQ ID NO: 42.

- 24. (New) The transformant according to claim 6,
 wherein the gene encoding propionate kinase encodes the following protein (a) or (b):
 (a) a protein comprising an amino acid sequence as shown in SEQ ID NO: 43; or
 (b) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 43 by deletion, substitution, or addition of one or 2-3 amino acid residues and having propionate kinase activity.
- 25. (New) The transformant according to claim 6, wherein the gene encoding propionate kinase comprises a nucleotide sequence as shown in SEQ ID NO: 44 or a nucleotide sequence that hybridizes under stringent conditions to a complement of SEQ ID NO: 44.